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REVIEW

The Vomeronasal System and Its Connections with Sexually Dimorphic Neural Structures

JORGE LARRIVA-SAHD and AKIRA MATSUMOTO[1]

Laboratory of Experimental Pathology, Instituto Nacional de la Nutrición SZ, Mexico City, Mexico and [1]Department of Anatomy, Juntendo University School of Medicine, Hongo, Tokyo 113, Japan

INTRODUCTION

The vomeronasal system in rodents plays an important role in modulating the sexual neuroendocrine and behavioral responses elicited by pheromones. This influence largely depends on the ability of the vomeronasal organ (VNO) to transduce chemical signals (i.e. pheromones) released from individuals of the same colony into receptor potentials which ultimately modulate the neural substrates controlling gonadotropin secretion. Since most if not all sexual endocrine and behavioral responses modulated by the vomeronasal system are sexually dimorphic and so are most of its central relay nuclei, this unit constitutes a model system in which morphological and functional sex differences are evident. Furthermore, this system has provided a better understanding of the mechanisms by which exteroceptive stimuli may influence mammalian reproductive functions. The present review concerns with the structure and function of the rodents VNO and emphasizes on the central pathways and sex differences of the vomeronasal system.

STRUCTURE AND EFFERENT CONNECTIONS OF THE VNO

(i) *Macroscopic anatomy*

The vomeronasal organ (VNO) (Organ of Jacobson) [53] is a paired, fusiform chemoreceptor. In the rat, it is approximately from 5 to 7 mm in length and 1.5 mm in diameter. Bilaterally located in the most ventral part of the nasal septum, the VNO contains a blind sac lined by two types of epithelia [32, 45, 112] (Fig. 1). In most mammals, the organ housed in an osseous chamber opens anteriorly by way of a narrow duct. The epithelial tube in some species opens ventrally to the mouth through the nasopalatine duct. The VNO in rodents and higher terrestrial mammals opens directly into the nasal cavity, just behind the nostrils [32]. The epithelium lies on highly vascularized loose connective tissue containing numerous serous glands and nerves (Fig. 2). The osseus shell of the VNO is pierced in its posterior third by branches of the vomeronasal nerves, which reach the accessory olfactory bulb (AOB) as they pass through the cribriform plate of the ethmoid bone. It is noteworthy that as the branches of the vomeronasal nerves leave the VNO, they become intimately associated with branches and ganglia of the terminal nerve. This association makes the terminal nerve prone to damage in experiments designed to study the effects of the removal of the VNO [94]. This should not be underestimated since like the VNO, the terminal nerve has also been implicated in the sexual responses elicited by olfactory signals [140, 141]. In addition to the vomeronasal nerves, the VNO receives autonomic and sensory innervation from the sphenopalatine and trigeminal ganglia [66, 73, 74, 119].

(ii) *Microscopic structure*

Although marked histological differences of the VNO have been documented in several species [32, 45, 112], three elements remain constant: a neurosensory epithelium, exocrine glands, and large venous sinuses [45, 112] (Figs. 1 and 2). In rodents, the vomeronasal epithelium lies medial to a large confluent venous sinus, the vomeronasal vein [130], for which the epithelial sac adopts a crescent moon shape. As mentioned, the lumen is lined by two different types of epithelium. In the concave (i.e. lateral) portion, there is a pseudostratified columnar epithelium with short microvilli and occasional cilia. The convex (i.e. medial) one is covered by a sensory neuroepithelium (Fig. 2). The former has been properly termed as receptor-free epithelium, instead of respiratory or ciliated, since its morphology is markedly different from that of these other epithelia [12]. Unlike most epithelia, the VNO neuroepithelium is devoid of basal lamina and possesses a capillary network (Fig 3), displaying distinct ultrastructural and probably functional characteristics [13, 88].

The VNO neuroepithelium is composed of three cell types: bipolar, supporting, and basal cells. Bipolar cells are chemoreceptors with a dendrite that runs apically and reaches the lumen and with an axon which leaves the neuroepithelium as it reaches the lamina propria. Cell nuclei of bipolars are round and located within the middle and basal third of the neuroepithelium [86]. Supporting cells also extend from the

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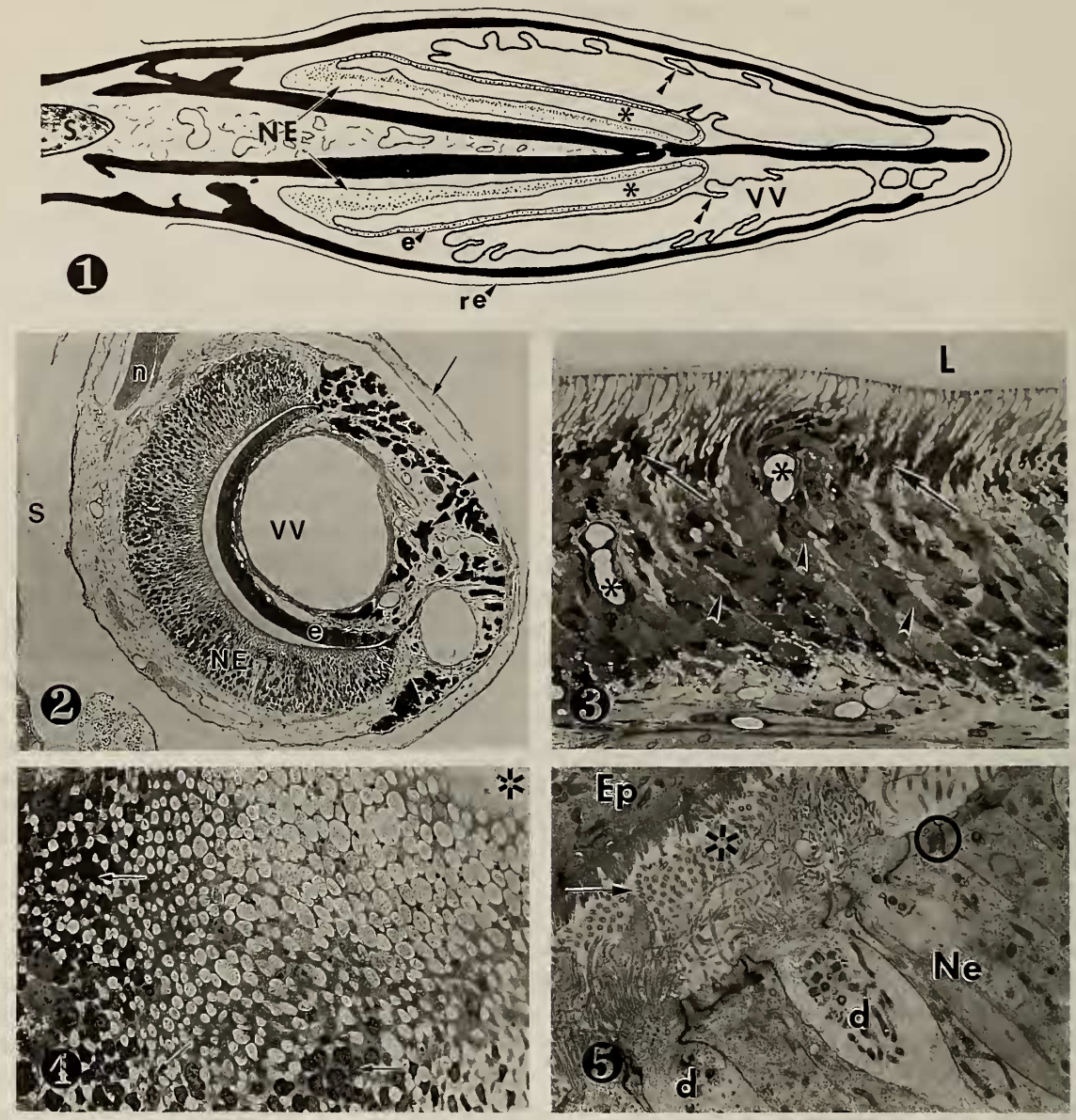

FIG. 1. Camera lucida drawing of an horizontal section of the rat vomeronasal organ. In the anterior part is identified by the cartilaginous nasal septum (S). On each side of the septum, the neuroepithelium (NE) is seen separated from the receptor-free epithelium (e) by the lumen (asters), more laterally the vomeronasal vein (VV) is also illustrated. The valves (arrowheads) project to the lumen of the vein. The organ is sequestered by bone (black), which is covered by the respiratory epithelium (re). Notice the association of the vascular and epithelial laminae which lie parallel to each other. 45×. Toluidine blue staining.

FIG. 2. Coronal section through the right vomeronasal organ. The organ is seen surrounded by bone, medially by the septum (S) and laterally by a thin bonny shell (arrow). The neuroepithelium (NE) shows its characteristic crescent-shape being separated from the receptor-free epithelium (e) by the lumen (no labeled), the vomeronasal vein (VV) lies laterally, in association with numerous serous acini (arrowheads). A non-myelinated nerve is seen in the lamina propria (n). 40×. Toluidine blue staining.

FIG. 3. Light micrograph of the rat vomeronasal neuroepithelium. Nuclei of supporting cells are oval and aligned in the inner part of the apical third whereas bipolar cell nuclei are evenly distributed within the basal area (arrowheads). The neuroepithelium contains blood capillaries (asters). Dendrites of bipolars project apically alternating with the basophilic processes from supporting cells (L=lumen). 300×. Toluidine blue staining.

FIG. 4. Micrograph from an horizontal section of the vomeronasal neuroepithelium. Most nuclei belong to supporting cells, whose basophilic cytoplasm surround dendrites which are somewhat paler. Notice that each dendrite is surrounded by supporting cells given a honeycomb appearance. 320×. Toluidine blue staining.

FIG. 5. Electron micrograph showing the apices of the receptor-free epithelium (Ep) and the neurosensory epithelium (Ne). Cells of the former send short microvilli to the lumen. 7000×.

lumen to the base of the epithelium, however these cells have smaller oval-shaped nuclei and remain within the luminal third [45]. Supporting cells show darker cytoplasm than that seen in bipolars (Fig. 3). Horizontal sections through the apical portion of the sensory epithelium reveal that supporting cells surround each dendrite of the receptor cell. Thus,

supporting cells provide a high degree of independence to each receptor dendrite (Fig. 4).

The apical part of the neuroepithelium has a number of membrane specializations [14]. Electron microscope shows that supporting cells alternate with dendrites of bipolar cells (Fig. 5). Dendrites of receptor cells send numerous thin and thick microvilli and occasionally cilia to the lumen, while supporting cells send only microvilli [86, 112]. Both cell types are united apically by tight junctions and desmosomes. Unlike the primary olfactory epithelium in which gap junctions occur between receptor cells, gap junctions in the VNO epithelium are only found between supporting cells [97, 98]. This may again reflect a high degree of functional individuality in the receptor cell.

Basal cells are confined to the base of the epithelium. These cells are devoid of processes and show occasional mitoses [134]. Contrary to earlier suppositions [102, 103], it is now accepted that basal cells actually represent the stem cell of the neuroepithelium which gives rise to supporting and receptor cells [4, 5]. This has been documented in experiments performed on adult mice, which show that following axotomy or removal of the AOB, basal cells incorporate thymidine and undergo mitosis. Thus, basal cells give rise to precursors which migrate and differentiate into mature bipolar and supporting cells [5, 6]. It is interesting to note that young receptor cells must have the potential of originating new axons, which by reaching the central nervous system achieve functional maturity. Furthermore, it has estimated that in the adult mouse, each new axon must travel at least 7 mm from the VNO to the first relay: the AOB [6]. This plasticity of VNO receptor cells has become even more interesting with the recent demonstration that VNO axons reach the medial preoptic area (MPOA) and hypothalamus [64]. These direct connections bring up the question of whether the newly-formed axons travel within the central nervous system to reach the diencephalon. In this case, the distance estimated by Barber [6] is at least twice long. Resolution of this issue is required to enhance our understanding of the plasticity of sensory cells.

(iii) *Sex differences in the VNO*

The existence of possible structural sex differences in the VNO is still matter of controversy. While several authors have reported a lack of sex differences in VNO size [32] or the neuroepithelium [14, 34], others have reported sex differences in neuroepithelial volume and nuclear size in bipolar and supporting cells [116, 117]. These results deserve to be taken into account, since if sex differences are present in the VNO neuroepithelium, it implies that perinatal titers of sex steroids could induce perinatally permanent changes on the cells of which (i.e. basal cells) undergo a process of cell division and differentiation [3] beyond the so-called critical period of development.

INTERACTION OF PHEROMONES WITH THE VNO

(i) *Extrinsic mechanisms of transport of odors*

Transduction of odors into receptor potentials is the main task of the VNO neuroepithelium [100]. The VNO is isolated from the nasal and oral cavities and its opening is exceedingly narrow [45]. Therefore, the process of transduction largely depends on the transport of a potential stimulus to the domain of the receptor cell [91]. Consequently, the VNO possesses an associated group of structures facilitating the transport of minute quantities of non-volatile material from the habitat to the lumen of the organ. On this premise, the transport could be divided into two systems: an extrinsic one located outside the VNO, and an intrinsic one within the VNO itself. Among the most illustrative extrinsic mechanisms is that used by garter snakes to internalize airborne stimuli. These reptiles capture environmental molecules by fast tongue flicking followed by a retraction of the tongue transferring airborne molecules to the opening of the VNO [17, 36, 44, 45]. In mammals, especially in ungulates and carnivores, the so-called "Flehmen" seems to be the counterpart [83]. This consists of coordinated movements of the neck, facial, and tongue muscles, which, in conjunction with the inspiratory flow of gases through the nasal cavity, displace particles attached to mucous and serous secretions from nostrils to vestibulum, thereby facilitating the entry of substances into the VNO. In laboratory animals, especially in guinea pigs, transport of low-volatility substances is achieved by the so-called "head-bobbing". This is preceeded by an investigative behavior leading to reach the urine of conspecifics, followed by fast, backward and forward head movements, varying from one to four cycles per second [7]. The head-bobbing culminates with the entry of non-volatile substances into the VNO lumen [143].

(ii) *Intrinsic mechanism of transport of odors*

According to Broman's early postulation, the VNO itself acts as a pump, taking up or ejecting fluids to or from the lumen [16]. In fact, once a potential stimulus has been brought to the VNO opening, the VNO promotes further transport towards the neurosensory epithelium on its own. Wysocki *et al.* [143] demonstrated that when hamster's urine is mixed with rhodamine, the fluorescent tracer eventually reaches the lumen of the VNO. More recently, autoradiographic studies have given additional evidence to the uptake of odors by the VNO [107]. Studies in cats [31] demonstrated that electrical stimulation of the cervical sympathetic nerve produced suction of fluid into the VNO lumen, whereas parasympathetic stimulation produced ejection of fluid from the VNO. This "pumping" seems to be due to the caliber of the VNO, which in turn is determined by the vasomotor activity of the autonomous nervous system [91, 92]. In fact, this displacement results from the amount of blood passing through the vomeronasal vein. Since the VNO epithelial tube lies in parallel to the vomeronasal vein [130] (Figs. 1 and 2), the volume of the VNO lumen is inversely related to the diameter of the vomeronasal vein. In summary, the auto-

nomic nervous system controls the access of non-volatile material to the VNO by influencing the rate of blood supplied to the vomeronasal vein [91].

Ciliary activity and secretions from the associated glands represent additional means of promoting transport and distribution of non-volatiles along the VNO lumen. In fact, ciliated cells of the receptor-free epithelium [13, 104], may contribute to the transport of substances along the epithelial surface. In addition, the serous glands lying in the lamina propria may also play a role in the process of transport of potential stimuli. Mendoza [87] has found that acini of the vomeronasal glands and surrounding smooth muscle fibers receive autonomic nerve endings. Therefore, it is likely that upon autonomic stimulation, serous secretory products are released to the lumen. These secretions are capable of distributing and rendering odors water soluble, which is a prerequisite for interaction with the receptor's plasma membrane [93].

Immunohistochemical studies have demonstrated the presence of some peptides in the lamina propria, blood vessels and receptor-free epithelium. These substances include galanin [73, 74], substance P [66], and calcitonin gene-related protein (CGRP) [119]. However, their possible functional implications remain to be elucidated. Recently, it has been proposed that because of its association with the sphenopalatine ganglion and nervous plexuses of the VNO, galanin may be involved in controlling the vasomotor activity of the VNO venous plexuses [74].

(iii) *Transduction of olfactory stimuli*

Once pheromones have reached their targets (i.e. receptor cells), the process of transduction from chemical stimulation to receptor potentials takes places. Most of the available information about the cellular events involved in the transduction of odors into action potentials derives from the main olfactory system. Hence, even though there are cytological and immunocytochemical differences between bipolar cells from the main and accessory olfactory system [23, 54, 85], there seems to be no reason to suppose that the transduction process in the VNO would be different [94]. On this premise, the microvilli of bipolar cells of the VNO are the counterpart of the cilia which comprise most of the transduction apparatus of primary olfactory receptor cells [70, 84]. In this place olfactory stimuli bind to the receptor cell membrane inducing an intracellular increment of cAMP or IP_3 via G-protein binding receptor molecules [10, 11]. Receptor potentials seem to be mediated by activation of non-specific cation channels [37] via cAMP-activated or IP_3-activated Ca^{++} channels [55].

EFFERENT CONNECTIONS OF THE VNO

(i) *The polysynaptic pathway*

Santiago Ramón y Cajal [19] demonstrated that the vomeronasal nerves project to dendrites of mitral cells of the AOB. These original observations have been confirmed by other researchers so it is accepted that the AOB is the first relay of the vomeronasal system. Apart from this direct projection, a short path from mitral cells to the granule cell layer has been proposed on the basis of quantitative autoradiography [3] and anterograde transsynaptic transport of horseradish peroxidase [51]. From the AOB fibers project centrally in the accessory olfactory tract to the bed nucleus of the accessory olfactory tract (BNAOT), medial amygdaloid nucleus (MAN) and posteromedial cortical amygdala (PMCAN), as well as to the posterodorsal part of the bed nucleus of the stria terminalis (BNST) [15, 114]. Since it was found that the MAN and PMCAN receive mostly, but not exclusively [69], direct projections from the AOB [51], these nuclei have been joined by the term "vomeronasal amygdala" [57]. Electrophysiological studies have typified the excitatory and inhibitory nature of these connections. Furthermore, this technique has also shown that the vomeronasal amygdala has reciprocal inhibitory and excitatory connections with both the AOB and hypothalamic ventromedial nucleus (VMN) [142]. Because of the implications of the amygdala in reproductive functions [82, 113], this circuitry may represent a substratum of the process of integration of olfactory inputs prior to reaching the diencephalic nuclei involved in sex behavior and gonadotropin secretion [142]. The vomeronasal amygdala has different outputs. While the MAN sends efferents to the medial preoptic-anterior hypothalamic area and ventromedial and ventral premammillary hypothalamic nuclei, the PMCAN projects to the retrocommissural BNST as well as to the contralateral MAN.

(ii) *The direct pathway*

Recently a monosynaptic link between the VNO and the MPOA, VMN, and hypothalamic arcuate nucleus (ARCN) has been found [64]. Thus, selective transection of the vomeronasal nerves inside the bonny capsule (Figs. 6 and 7) produces orthograde degeneration in the neuropil of these nuclei, demonstrating that axons of bipolar cells do end up in the neuropil of these nuclei. Although this approach precluded proving whether vomeronasal efferents project on luteinizing hormone-releasing hormone (LHRH) producing neurons, it is conclusive that bipolar cells from the VNO can have direct access to the nuclei in which LHRH neurons have been encountered [21, 33, 60, 118]. Therefore, it is likely that through this direct pathway the excitatory and inhibitory receptor potentials generated in bipolar cells may reach the MPOA and medial basal hypothalamus, influencing sex behavior and gonadotropin secretion in a monosynaptic fashion (Figs. 8 to 12).

SEXUALLY DIMORPHIC NUCLEI RECEIVING INPUTS FROM THE VNO

The VNO projects to some of the brain areas involved in the control of gonadotropin secretion and sex behavior displaying structural sex differences [40, 45, 71, 117]. These nuclei will be reviewed next.

(i) *Sex differences in the neuroendocrine brain at light microscopic level*

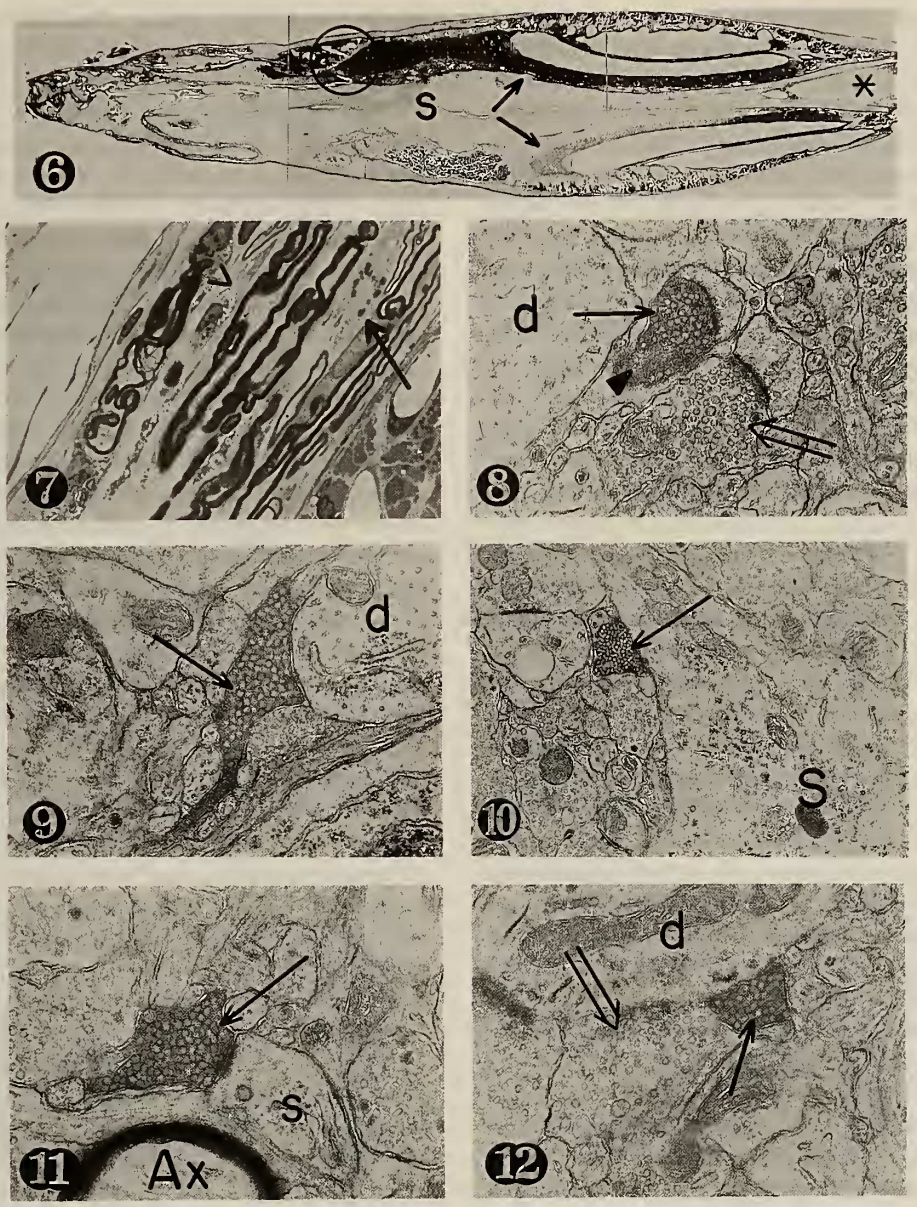

FIG. 6. Horizontal section of the vomeronasal organ. The cartilage of the nasal septum can be seen in the anterior part (aster). The neuroepithelium (arrows) is seen on both sides of the septal bone (S). As shown the lesion (circle) is circumscribed to the lumen, which is encased by the bonny shell. 40×. Toluidine blue staining. (Reproduced with permission from Ref. [64]).

FIG. 7. Light micrograph of a branch of the vomeronasal nerve caudal to the cut. The myelinated nerves have a waving contour and some Schwann cells display vacuolated cytoplasm (arrow head). A mitotic spindle of a Schwann cell is seen (arrow). 480×. Toluidine blue staining. (Reproduced with permission from Ref. [64]).

FIG. 8. Electron micrograph of the neuropil of the central part of the MPOA of a rat with transection of the vomeronasal nerves. Two axodendritic spine synapses are seen, a degenerating one (single arrow) whose matrix is very electron-dense, containing numerous round-shaped vesicles and a mitochondrion (arrow head). Another bouton of normal appearance is seen (double-tailed arrow). In the upper left a dendritic shaft (d) is observed. 15,000×. (Reproduced with permission from Ref. [64]).

FIG. 9. Electron micrograph showing a degenerating terminal (arrow) contacting with a dendritic shaft (d) in the neuropil of the central part of the MPOA. 15,200× (Reproduced with permission from Ref. [64]).

FIG. 10. Micrograph of the central part of the MPOA in which a degenerating bouton makes an axosomatic contact (S = neuronal soma). 7,000 × (Reproduced with permission from Ref. [64]).

FIG. 11. Neuropil of the VMN showing orthograde degeneration (arrow). The bouton is synapsing a dendritic spine (s). At the bottom a myelinated axon (Ax) is seen. 15,200× (Reproduced with permission from Ref. [64]).

FIG. 12. Electron micrograph of the neuropil of the ARCN. A degenerating terminal (single arrow) is seen in contact with a dendritic shaft (d). A normal appearance synaptic bouton (double arrow) is also seen forming an axodendritic synapse. 15,000× (Reproduced with permission from Ref. [64]).

In 1978, Gorski *et al.* [39] reported a marked sex difference in the MPOA of the rat brain. The volume of this intensely stained neuron group, which was termed as sexually dimorphic nucleus of the POA (SDN-POA), is markedly larger in the male than in the female. Sex difference in nuclear volume is also found in the AOB of rats [24, 110, 116], in the BNST of rats and guinea pigs [49, 50] in the BNAOT [24, 25], VMN [77], in the MAN of rats [50, 99]. According to Del Abril *et al.* [28], no sex difference exists in the total nuclear volume of the BNST, but a sexual difference is found in the posterior region of the medial BNST. In general, it is assumed that the volume of these nuclei is larger in males than in females, and that neonatal castration of the male reduces the volume of these nuclei to the size of the female's. Conversely, the volume of the anteroventral periventricular nucleus (AVPV) of the POA is larger in female rats and guinea pigs than that in males [8]. Furthermore, in female rats [52] and guinea pigs [18] the volume of the AVPV decreases with perinatal exposure to androgen. In addition, it has been demonstrated that the nuclear volume of the anterior region of the medial BNST is greater in female rats than that in males, and is likewise modified by perinatal exposure to androgens [28]. These findings strongly suggest underlying sex differences in the number of neurons, afferent fibers and synapses as well as in the dendritic morphology, which ultimately account for the difference in nuclear volume. This postulated is supported by two facts. There is a difference in neuron density in the AOB [132], BNAOT [24, 25], BNST [42], AVPV [124], and MPOA [39] between sexes, and the difference in neuron density is determined by sex steroids in the perinatal period. As far as neuron processes are concerned, differences have been documented in dendritic morphology of AOB neurons in rats [20], and POA neurons in hamsters [41], monkeys [2], rats [46] and ferrets [22, 131]. Since studies of Weisz and Ward [138] indicate that plasma titers of androgen are much higher in males than in females, the observed sex differences in neuron density cannot be attributed only to genomic sex differences. This is further supported by the fact that suppression of endogenous gonadal sex steroid (i.e. testosterone) by perinatal castration of the male produces a feminine phenotype in terms of nuclear volume, neuronal density and dendritic morphology.

In 1985, Swaab and Fiers [129] reported a sexual dimorphism in the POA, in an area that appears to be the human counterpart of the SDN-POA [39]. Soon afterward, Allen *et al.* examined the human POA and subdivided the interstitial nucleus of the anterior hypothalamus (INAH) into four neuron groups: INAH 1–4 [1]. They found that the volume of both INAH-2 and INAH-3 is larger in men than in women, and argued that Swaab and Fliers's SDN-POA is the equivalent of their INAH-1 but did not detect significant sex difference in the INAH-1 volume. LeVay [68] reported that the INAH-3 was the only nucleus displaying sexual dimorphism. Although further studies are necessary to reconcile these results, it is worthwhile to note that sexual dimorphism is found in neuronal structures which are thought to be responsible for sexual functions [127]. It has now been demonstrated that during the early stages of development of the central nervous system, plasma levels of androgen are much higher in male than in female fetuses [139]. Therefore, it is conceivable that the male's higher plasma titers of perinatal androgen may in fact induce the difference in the nuclear volume of the POA in humans.

(ii) *Ultrastructural sex differences in the VNO pathways and neuroendocrine brain*

Quantitative electron microscopic studies have disclosed numerous sex differences in the synaptic organization of the mammalian brain [29, 78, 81]. Most efferent nuclei of the vomeronasal system are sexually dimorphic in terms of synaptic inputs. The number of axodendritic shaft synapses in the MAN of male rats is significantly greater than that of females [105]. In the ARCN, the number of axodendritic spine synapses in female rats is approximately two-fold greater than that in males, whereas the number of axosomatic synapses in females is approximately half of that in males (Table 1) [75, 76]. However, no sex difference was detected in the number of shaft synapses. This sexually dimorphic pattern of synaptic distribution is similar to that found in the strial part of the POA [108, 109]. More recently it was determined that in the

Table 1. Number of axodendritic and axosomatic synapses in the arcuate nucleus (ARCN) of normal and neonatally androgenized or castrated rats

Group	Number of rats	Axodendritic synapses[a]		Axosomatic synapses[b]
		Shaft synapses	Spine synapses	
Normal females	8	1655±94*	242±30	2.11±0.15 (166)[c]
Androgenized females	7	1507±79	174±31	3.84±0.26 (130)
Normal males	7	1607±138	144±24	3.86±0.20 (160)
Castrated males	7	1462±62	257±24	1.97±0.25 (150)

Reproduced with permission from Ref. [75]

* Mean±SEM.

[a] Axodendritic synapses were counted per 18,000 μm^2 in the ARCN.

[b] Number of axosomatic synapes per cell body. For counting synapes, only cell bodies whose profiles could be seen were randomly selected.

[c] Number of neurons in parenthesis.

MPOA the number of shaft synapses was higher in males than in females, while no sex difference was found in the number of spine synapses [63]. In the suprachiasmatic nucleus, the incidence of spine synapses is higher in males than in females [43, 65]. As previously proposed, the sexual dimorphism in neural connectivity [30, 111] may be due to a sexually differentiated synaptic population under the influence of perinatal sex steroid environment. All of these nuclei except the suprachiasmatic nucleus contain a number of sex steroid-accumulating neurons. Given the fact that with exception of the suprachiasmatic nucleus, all of these sexually dimorphic nuclei contain sex steroid-accumulating neurons [106, 128], these sex differences strongly support the idea that synaptic organization may vary according to the genomic responses of each individual nucleus to organizational action of sex steroids.

Although quantitative studies are required to determine whether the observed light microscopic sex differences in the AOB and BNAOT (vide supra) are underlain by ultrastructural difference(s), it is predictable that AOB and BNAOT may have quantitative sex differences in their synaptic organization. However, this hypothesis needs to be investigated.

According to Stumpf [128], Pfaff and Keiner [106] and Simerly *et al.* [126], there is a regional difference in the distribution pattern of sex steroid-accumulating neurons in the VMN. This correlates with a regional difference in the synaptic pattern in the VMN [78, 79, 80]. As shown in Figure 13, the number of shaft and spine synapses in the ventrolateral part of the VMN (VL-VMN), which contains abundant sex steroid-accumulating neurons, is significantly greater than that in the dorsomedial VMN (DM-VMN), which only contains a few. The number of shaft and spine synapses in the male VL-VMN is significantly greater than in the female VL-VMN, but no sex difference is found in the DM-VMN. These findings indicate that the presence of sexually dimorphic synaptic organization is only restricted to the VL-VMN which contains a number of sex steroid-accumulating neurons. Neonatal castration of the male reduces the number of shaft and spine synapses in the VL-VMN to a level comparable to the normal female. The number of shaft and spine synapses in the female VL-VMN is increased to the level comparable to that of normal males by neonatal exposure of androgen. These findings reinforce the significance of the sex steroid environment at neonatal period for the development of sexually dimorphic synaptic organization in the neuroendocrine brain.

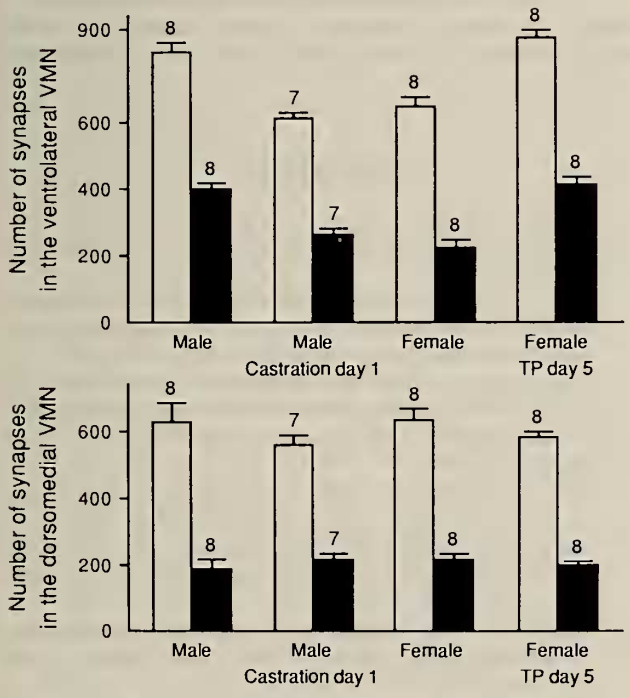

FIG. 13. Number of axodendritic shaft and spine synapses in the ventrolateral and dorsomedial parts per 10,000 μm^2 in the VMN of normal males (Male) and females (Female), males castrated on day 1 (Male Castrated day 1) and females treated with 1.25 mg testosterone propionate (TP) on day 5 (Female TP day 5). Open bars=shaft synapses; solid bars=spine synapses. Vertical lines indicate SEM. Number of vertical lines refer to the number of rats examined. (Reproduced with permission from Ref. [79]).

(iii) *Immunohistochemical analysis of the sexual differences in the neuroendocrine brain*

Recent immunohistochemical studies have shown that there is sexual dimorphism in the distribution pattern of neuronal cell bodies and fibers containing several types of neurotransmitters and neuromodulators. These include vasopressin in the BNST and POA [26, 133, 135], substance P in the BNST and MAN [26, 27, 72], cholecystokinin in the POA, BNST and MAN [62, 95, 96, 125], opioid peptides in the periventricular POA and MPOA [35, 124, 125, 137], galanin in the MPOA [9, 89, 90], calcitonin gene-related peptide (CGRP) in the MPOA [47, 48] LHRH in the POA [58, 59, 60], serotonin in the MPOA [120, 121], and tyrosine hydroxylase (TH) in the AVPV [122, 123].

The distribution pattern of vasopressin-immunoreactive fibers in the BNST [135], enkephalin-immunoreactive fibers in the periventricular POA [136, 137], and serotonin-immunoreactive fibers in the MPOA [120, 121] is also sexually dimorphic, and the distribution of these neurotransmitters depends on perinatal exposure to sex steroids. Similarly, some neuronal groups of the neuroendocrine brain bear immunohistochemical characteristics which are determined by the perinatal influence of gonadal sex steroids. Thus, the number of CGRP-immunoreactive neurons in the MPOA is significantly larger in male rats than that in females [47, 48]. Furthermore, neonatal castration of the male rat reduces the number of these neurons to that observed in the female. Conversely, neonatal exposure of the female rat to androgen induces a masculine pattern in the density of CGRP-immunoreactive neurons. On the other hand, the number of TH-immunoreactive neurons in the AVPV is larger in the female rat than in the masculine homologue [122, 123]. Furthermore, recent in situ hybridization histochemistry revealed that there is a larger number of TH mRNA expressing

neurons in the AVPV of the female or neonatally castrated male than in the intact male or neonatally androgenized female [126]. According to Merchenthaler *et al.* [90], the number of LHRH-immunoreactive neurons coexpressing galanin in the MPOA/diagonal band of Broca is 4 to 5 times higher in female rats than that in males. Moreover, neonatal castration of the male reduces the incidence of galanin-LHRH co-localization to that found in the female. These sex differences in the number of CGRP- and TH-immunoreactive neurons represent a unique aspect of sexual differentiation in the sense that only certain phenotypic characteristics of particular neuron lineage are dimorphic.

The LHRH neurons of the POA represent the final common pathway regulating gonadotropin secretion by the anterior pituitary. The activity of LHRH neurons is considered to be regulated by intra- and extrahypothalamic neuronal elements as well as the steroidal environment [33]. Since LHRH neurons do not accumulate estrogen [118], it seems plausible that other estrogen-sensitive interneurons in the POA may integrate steroidal signals which in turn modify the activity of LHRH neurons. Immunohistochemical studies have suggested that other neurochemicals may also modulate estrogen receptor (ER)-immunoreactive neurons in the POA [56]. This is supported by immuno-electron microscopic studies indicating that neuropeptide Y-[56], LHRH-[60], and enkephalin-immunoreactive terminals [61] make synaptic connections with ER-immunoreactive neurons. Langub and Watson [61] have pointed out that in female rats more enkephalin-immunoreactive terminals (synaptic and non-synaptic) contact the cell body of ER-immunoreactive neurons than those in males. Neurons located in the POA may be influenced by both estrogen and neurotransmitters/neuromodulators via, respectively, nuclear receptors and synaptic inputs. With respect to the LHRH neuronal system, a sexual dimorphism exists in the synaptic inputs to LHRH neurons in the rat POA [21]. Thus, LHRH neurons in females have approximately twice the number of synapses as do those of males. β-Endorphin-immunoreactive terminals contribute to this dimorphism. Because a subset of β-endorphin-immunoreactive neurons has been reported to accumulate estrogen [101] and to contain immunoreactive ER [67], it is possible that physiological differences in the regulation of gonadotropin secretion may also be reflected in a sexually dimorphic connectivity of the LHRH system in which this neuronal subset may be involved.

CONCLUDING REMARKS

While it is well established that in mammals the interaction of gonadal sex steroids with the central nervous system and pituitary represents the main regulatory stimulus of gonadotropin secretion [38, 82], studies carried out during the past few decades support the assumption that certain olfactory stimuli are capable of exerting a modulatory effect upon the neural structures which control gonadotropin secretion. Furthermore, it has been shown that in rodents pheromones modify gonadotropin secretion via the vomeronasal system. In fact, certain pheromones released in excretory and secretory products influence in the endocrine and behavioral aspects of reproductive function. The interaction of pheromones with the vomeronasal neurosensorial epithelium modifies gonadotropin secretion influencing onset of puberty, estrous cycle, gestation, feminine and masculine sexual behaviors, and maternal behavior. It is interesting to highlight that regardless of the pathway that nerve impulses follow from the VNO to reach the brain, terminations in the vomeronasal amygdala and medial preoptic-hypothalamic nuclei are the neuronal structures whereby pheromones may modify the sex-steroidal responses governed by the brain. Moreover, since most of these brain areas contain neurons with sex steroid receptors and display quantitative structural and biochemical sex differences, it is plausible that these characteristics may represent the inherent ability of individuals from each sex to respond differently to pheromonal stimuli. This postulate is made on the assumption that the same stimulus placed in the VNO should produce a receptor potential of equal magnitude regardless of sex, as it has been demonstrated by stimulation with some putative pheromones [100].

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